IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:	Confirmation No: 2174
Del Soldato *et al.*	Group Art Unit: 1617
Application Serial No.: 09/147,770	Examiner: Mitchell, G.W.
Filed: April 28, 1999	Attorney Docket No: 026220-00031

For: Nitric Ester Derivatives and Their Use in Urinary Incontinence and Other Diseases

March 16, 2005

* * * * * *

RESPONSE TO SECOND RESTRICTION REQUIREMENT

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the second restriction requirement dated February 24, 2005, the applicants elect:

Group VII, claims 1, 2, 9, and 10, and the species of claim 9. Claims 1, 2, 9, and 10 read on the elected species.

These elections are made with traverse for the following reasons:

1) The present application is U.S. national stage of PCT/EP97/04774 and is governed by the PCT unity of invention standard and rules. As indicated in the applicant's traversal of July 17, 2000 to the first restriction requirement, no objection as to unity of invention was raised during the International stage of this application. Under M.P.E.P. §1850, the examiner must follow PCT Rules 13.1 and 13.2 and may not issue a restriction requirement under 35 U.S.C. §§121 or 372.
2) The inventive concept relates to the NO_2 group attached to the compound through the bivalent connecting bridge X_1.

3) All the compounds of groups IIA) and IIIA) belong to the same therapeutically class *i.e.*, anti-inflammatory nonsteroidal drugs containing –CH(CH_3)-COOH (arylpropionic acid derivatives). Therefore, the compounds have common chemical structures and similar pharmacological activities.
4) The use of anti-inflammatory nonsteroidal drugs in urinary incontinence is reported at page 4 of the specification (WO 98/09948) and as described in Pharmacological Review, 1993, 45, 253-308 cited at page 3 of the specification (relevant pages enclosed). In view of the evidence for a role of prostanoids in the bladder disorders, the anti-inflammatory nonsteroidal drugs interfering with their synthesis are potentially useful in urinary incontinence.
5) The application as filed reports several pharmacological examples related to the activity in the urinary incontinence (Examples 1 to 15) showing that the compounds of the present invention have a better pharmacological activity than the corresponding parent drug.

Compounds	Pharmacological examples
(NO-I) - NO-Indomethacin (R = group IV A)	1, 5, 7, 8, 10, 11, 13
(NO-N) - NO-Naproxen (R = group III A)	2, 14

6) Finally, the two declarations of Dr. Piero Del Soldato were submitted in order to demonstrate unexpected results for the compound of claims 9 and 26.

The applicants respectfully submit that this application is generally in condition for allowance and request favorable action thereon. Specifically, the subject matter in claims 9 and 10 was previously indicated as allowable. Further, the applicants respectfully draw the examiner's attention to claim 26, which is not the subject of any rejections. The applicants believe that this claim is also allowable.

This response is filed within the shortened statutory period for response, no fee due. In the event this response is not timely filed, the applicants hereby petition for an appropriate extension of time. The fee for this extension, along with any additional fees

required with respect to this response, may be charged to Deposit Account No. 01-2300, referencing Attorney Docket No. 026220-00031.

Respectfully submitted,

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PHARMACOLOGICAL REVIEWS
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Pharmacology of Lower Urinary Tract Smooth Muscles and Penile Erectile Tissues*

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Appendix A

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ACE is not the sole enzyme able to convert AtI to AtII ornish et al., 1979; Oliver and Sciacca, 1984). In dog lated renal artery without endothelium, the AtI-in-ced relaxation was not significantly attenuated by E inhibition, suggesting that AtII-generating en-ne(s) other than ACE exist in vascular tissue (Okun-i et al., 1984; Okamura et al., 1990). This may be the e also in the human detrusor. Further experiments on lated membranes from the human detrusor have own the presence of an enzyme (or enzymes) other n ACE that is able to convert AtI to AtII, most bably a serine proteinase (Lindberg et al., 1993). Such enzyme (or enzymes) has been demonstrated to con-t AtI to AtII in the human heart in the presence of topril (Urata et al., 1990a,b).

revious investigations (van Sande et al., 1985) dem-trated the occurrence of ACE in the human bladder. amounts were low compared to the levels found in er parts of the urogenital tract, e.g., the normal pros-. Notably, very high concentrations were found in hypertrophic prostate (Yokoyama et al., 1980, 1982; Sande et al., 1985). Because bladder hypertrophy is mon in patients with benign prostatic hyperplasia, annot be excluded that the bladder renin-At system be different from normal in this condition. Further ies on the effects of AtI and AtII on human bladder cle, as well as on the characteristics of possible AtII-rating enzymes other than ACE, in normal and rtrophic human bladder seem necessary.

d. PROSTANOIDS. The importance of prostanoids in the regulation of detrusor function has attracted considerable interest, and the topic has been the subject of several reviews (Andersson and Forman, 1978; Andersson and Sjögren, 1982; Pavlakis et al., 1983; Mikhailidis et al., 1987; Maggi, 1992). Prostanoids are known to be generated locally by physiological stimuli such as stretch of the detrusor smooth muscle (Gilmore and Vane, 1971; Ghoneim et al., 1976; Poggesi et al., 1980) but also injuries of the vesical mucosa (Downie and Karmazyn, 1984), nerve stimulation (Khalaf et al., 1979; Alkondon and Ganguly, 1980; Dveksler et al., 1989), and agents such as ATP and mediators of inflammation, e.g., BK (Nakahata et al., 1987; Maggi et al., 1989d), and the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (Giuliani et al., 1991) may initiate prostanoid generation.

Both during contraction (Bultitude et al., 1976; Khalaf et al., 1980; Klarskov, 1987c) and under basal conditions (Brown et al., 1980; Poggesi et al., 1980), E-type PGs were found to be synthesized and released by the bladder of various species. That the major prostanoid produced by the rabbit bladder was PGE_2 was confirmed by Leslie et al. (1984). They also showed that the rabbit bladder was capable of releasing PGI_2, $PGF_{2\alpha}$, and TXA_2. In rat bladder, on the other hand, PGI_2 was the principal prostanoid, with smaller amounts of PGE_2 and TXA_2 being produced (Jeremy et al., 1984). Biopsies of human urinary bladder mucosa were demonstrated to release the same prostanoids as the rabbit bladder, but the quantitative order was $PGI_2 > PGE_2 > PGF_{2\alpha} > TXA_2$ (Jeremy et al., 1987). Obviously, there seems to be marked species variation in the spectrum of prostanoids and their relative amounts synthesized and released by the urinary bladder (Larsson, 1980).

Several investigators have shown that $PGF_{2\alpha}$, PGE_1, and PGE_2 contract isolated human, as well as animal, detrusor muscle, whereas $PGF_{2\alpha}$ contracts and PGE_1 and PGE_2 relax or have no effect on the urethra (Ambache and Zar, 1970; Abrams and Feneley, 1976; Persson, 1976; Andersson et al., 1977, 1978b; Khanna et al., 1978; Khalaf et al., 1979; Klarskov et al., 1983a; Maggi et al., 1984a, 1985a, 1988b; Ueda et al., 1985; Gotoh et al., 1986a; Poli et al., 1992a). Misoprostol, a stable PGE_1 derivative, had no effect on human bladder, whereas other derivatives of this PG had contractile effects comparable to those of the parent compound (Poli et al., 1992b). Prostanoids may affect the excitation-contraction coupling in the bladder smooth muscle in two ways: directly by effects on the smooth muscle and/or indirectly via effects on neurotransmission.

The membrane potential of guinea pig and rabbit smooth muscle cells was unchanged by low concentrations of PGE_2 (up to 10^{-6} M), but higher concentrations depolarized the cells and increased the frequency of spontaneous action potentials. It was concluded that

prostanoids are not normally released by the nerves to the guinea pig urinary bladder but are able to facilitate excitation-contraction coupling, possibly by mobilizing Ca^{2+} (Creed and Callahan, 1989). The response of bladder smooth muscle to prostanoids is slow, and it is unlikely that these agents are directly involved in the evacuation of the bladder by exerting direct effects on the bladder smooth muscle (Andersson and Sjögren, 1982). The prostanoids may affect the excitation-contraction coupling in lower urinary tract smooth muscle by indirect effects, leading to a modulation of the efferent and/or afferent neurotransmission. When physostigmine was added to the rabbit bladder, spontaneous contractions and basal tone increased. Because these effects were inhibited by indomethacin, and the inhibition was counteracted by $PGF_{2\alpha}$, it was suggested that there was a connection between the effects mediated by acetylcholine and by prostanoids in the bladder (Bultitude et al., 1976). This suggestion was supported by Borda et al. (1982), who found that, in the human bladder, endogenous prostanoids facilitated the action of acetylcholine through an increase in its concentration, probably by an inhibition of the acetylcholinesterase activity. Johns and Paton (1977) found that contractions elicited by transmural electrical stimulation of rabbit and monkey detrusor were moderately reduced when the preparations were exposed to atropine or indomethacin but that the inhibition was marked when both agents were present simultaneously. The potential NANC transmitter ATP is degraded to ADP, which causes release of prostanoids. In turn, these may contribute to tone, spontaneous contractions, and modulation of excitatory neurotransmission (Choo and Mitchelson, 1977; Dean and Downie, 1978b; Andersson et al., 1980; Husted et al., 1980b,c; Downie and Larsson, 1981; Anderson, 1982; Kasakov and Vlaskovska, 1985).

Available results thus suggest a role for the prostanoids as modulators of efferent neurotransmission.

Capsaicin-sensitive afferents in the bladder are chemosensitive and can be activated by prostanoids to increase the afferent input produced by a given degree of bladder filling. Maggi et al. (1988b) and Maggi (1992) suggested that prostanoids may be the link between detrusor muscle stretch produced by bladder filling and activation of capsaicin-sensitive afferents by bladder distension. Evidence for this was produced in the rat urinary bladder, where intravesical instillation of PGE_2 lowered the threshold for reflex micturition, and topical application of PGE_2 and TXB_2 on the serosal surface activated reflex micturition. Both effects were prevented by systemic capsaicin desensitization. Indomethacin pretreatment and systemic capsaicin increased the micturition threshold without affecting the amplitude of the micturition contraction. Because intravesical PGE_2 did not reduce the residual urine volume in capsaicin-pretreated animals, it was suggested that endogenous prostanoids enhance voiding efficiency through an action, direct or indirect, on sensory nerves (Maggi et al., 1988b; Maggi, 1992).

Prostanoids may also be involved in the pathophysiology of different bladder disorders. As pointed out by Maggi (1992), in cystitis there may be an exaggerated prostanoid production leading to intense activation of sensory nerves, increasing the afferent input. In addition, through the "efferent" function of capsaicin-sensitive bladder afferents, there may be a neurogenic contribution to the overall inflammatory process. Supporting this view, Abelli et al. (1992) found that SP injected intravenously in rats, via stimulation of NK_1 receptors, promoted plasma exudation in the urinary bladder by release of cyclooxygenase metabolites of arachidonic acid. This release may be caused by SP interacting with mast cells and was suggested to amplify the direct effect of SP on the permeability of postcapillary venules.

In view of the evidence for a role of prostanoids in the function of the lower urinary tract, it is not surprising that these agents, and drugs interfering with their synthesis, have been used therapeutically (Andersson, 1988). Bultitude et al. (1976) gave PGE_2 intravesically to women with difficulties in micturition and with various degrees of urinary retention. They found a decrease in the bladder volume at which voiding was initiated, an increase in bladder pressure, and a decrease in residual urine in two-thirds of their patients ($n = 22$). Most interestingly, the effects were reported to last for several months. These results were confirmed in a prospective study by the same group (Desmond et al., 1980). Also, other investigators have found beneficial effects of intravesical prostanoids in, for example, patients with neurogenic bladder dysfunction (Vaidyanathan et al., 1981c) and patients with partial or complete urinary retention after surgery for female stress incontinence (Tammela et al., 1987). However, other investigators have not demonstrated any such effects, either in patients with chronic retention (Delaere et al., 1981) or in postoperative patients (Wagner et al., 1985). Both PGE_2 and sulprostone (a derivative of PGE_2), instilled intravesically, caused a strong urgency sensation, resulting in reduced bladder capacity and bladder instability (Schüssler, 1990).

Thus, the clinical value of prostanoids in the treatment of urinary retention is controversial. If prostanoids are involved in the genesis of bladder hyperactivity, use of inhibitors of their synthesis would be a logical treatment alternative. In the few controlled studies published, both flurbiprofen (Cardozo et al., 1980) and indomethacin (Cardozo and Stanton, 1980) produced symptomatic relief in patients with bladder instability but had little effect on urodynamic parameters, and the incidence of side effects was high.

e. 5-HYDROXYTRYPTAMINE (SEROTONIN). 5-HT has been shown to contract the bladder or isolated bladder smooth muscle from several species (Gyermek, 1962;

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